THE EFFECTS OF SYNTHETIC GLUCOCORTICOSTEROID (TRIAMCINOLONE DIACETATE) AND DIBUTYRYLADENOSINE-3′,5′-(CYCLIC) MONOPHOSPHATE ON HEPATIC LIPASE ACTIVITIES IN RATS*

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1. Introduction

Recently glycogenolytic and gluconeogenic effects of glucagon in rats have been extensively investigated and are believed to be mediated in both cases, by cyclic AMP [1, 2]. The increased lipolysis in adipose tissue noted after the addition of glucagon and catecholamines involves the activation of a tissue triglyceride lipase and is believed to be mediated by cyclic AMP [3, 4]. Wieland and coworkers [5] using liver perfusion techniques, provided direct evidence for similar effects of glucagon and cyclic AMP on glucose and ketone body production by livers from 24 hr fasting rats. They suggested that the action of the hormone in the liver was similar to its action in adipose tissue, and involved the activation of a hepatic lipase. Similar conclusions have also been arrived at by other investigators [6, 7], using slices and homogenates from fed and 24 hr fasting rats.

Pyruvate carboxylase is a key enzyme in gluconeogenesis and has an absolute requirement for acetyl-CoA [8]. Acetyl-CoA is a product of fatty acid degradation (β-oxidation) and the release of fatty acids from triglycerides by tissue lipases therefore, is considered as an important step in the reaction sequences in gluconeogenesis. Recently Wieland and coworkers [9] demonstrated the occurrence in rat liver of two (possibly three) lipases. The ones occurring in the mitochondria and microsomes have pH optima at 5.0 and 8.5 respectively, and the one which is found in the high speed supernatant fraction has an optimum pH at 8.5. This latter one is believed to be the same enzyme found in the microsome. These lipases do not require heparin for activation. In the present study, an investigation was conducted to determine whether the elevation of blood glucose levels observed in fasting rats after the administration of glucocorticosteroids also involves the activation of one or more of the hepatic lipases, and whether cyclic AMP can elicit the action of glucocorticosteroid hormones. The results are presented herein. The heparin-activated lipase in rat liver was not included in this study.

Abbreviation:
BcAMP: $N^{6'},O^{2'}$-dibutyryl adenosine 3′,5′monophosphate.

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2. Materials and methods

For the present study, rats of 120 to 130 g body weight and of the strain previously reported [10], were provided with water and Purina rat chow *ad libitum*. The details regarding treatment with hormone (triaminolone diacetate), method for the preparation of mitochondria, microsomes, and high-speed supernatant fraction, and other conditions of the experiment were the same as described in previous papers [10, 11]. The method for the assay of lipase activity at pH 5.0 and 8.5 was the same as described by Wieland et al. [9]. The animals maintained on food (Purina rat chow) and water or no water alone, were sacrificed after 48 hr.

3. Results

Among the three hepatic lipases, the one occurring in the mitochondria (in fact is found in lysosomes [12]) and has an optimum pH at 5.0 is more active and more affected by fasting for 48 hr than the one occurring in the high-speed supernatant fraction (table 1). The lipase activity in the mitochondria, unlike that of the one found in high-speed supernatant fraction, is further decreased if fasting is continued for 96 hr. The lipase activity in the microsomes is not affected under these conditions (table 1).

A drop in blood glucose level by 25–30% and complete depletion of hepatic glycogen are found in rats after 48 hr fasting [11]. The administration of triamcinolone diacetate (a highly potent synthetic glucocorticosteroid) to such (48 hr fasting) rats 16 hr prior to sacrifice, restores the lowered blood glucose levels and lipase activities to their respective normal or near normal levels (table 2). The lipase activity in the mitochondria is increased more than the activity of the lipase in the high-speed supernatant fraction. Much larger increase in lipase activity at pH 7.4 was observed by Bewsher and Ashmore [7] in liver homogenate from glucagon-treated rats. When hormone is administered to 48 hr fasting rats 4 hr prior to sacrifice, lipase activities at pH 5.0 and 8.5 are not changed, but blood glucose levels are considerably elevated (table 2). Neither blood glucose levels nor the activities of hepatic lipases are affected when BcAMP is administered 16 hr prior to sacrifice. However, when administered 4 to 6 hr prior to sacrifice, blood glucose levels are elevated but lipase activity at pH 5.0 or 8.5 is not affected. The increase in lipase activity and the elevation of blood glucose level observed 16 hr after hormone administration, can be blocked by actinomycin D and cycloheximide. The results suggest that the observed increases in lipase activities have all the earmarks of a true induction process involving an increase in the rate of enzyme synthesis (table 2).

Triamcinolone diacetate (0.02 to 1.0 mg/ml), cyclic AMP or BcAMP or 5′-AMP (0.05 to 1.0 μmoles/ml) when added to the assay system containing mitochondrial lipase or lipase from high-speed supernatant fraction, does not increase their activities, but when added at higher concentrations (2 to 5 μmoles/ml) cyclic AMP, BcAMP, and 5′-AMP inhibit lipase activities. The inhibitions observed at pH 5.0 and 8.5 are between 30 to 40% and 15 to 20% respectively. This indicates that mitochondrial lipase activity is more affected than the one in the high-speed supernatant fraction (table 3). Stimulating effect at lower concentrations and inhibitory effect at higher concentrations of cyclic AMP on lipase activity at pH 7.4 were noted by Rizack [4] in a cell-free lipolytic system from adipose tissue.

Table 1
Effects of fasting on blood glucose levels and hepatic lipase activities in rats.

Animal	Lipase activity* in mitochondria (pH 5.0)	Lipase activity in microsomes (pH 8.5)	Lipase activity in soluble fraction (pH 8.5)
Control	4.84	1.42	2.62
Fasted for 48 hr	2.92	1.48	1.96
Fasted for 96 hr	2.38	1.39	1.94

* Lipase activity is expressed as μmoles of fatty acids released/g of liver/30 min.

Glucose in blood was estimated by the method of Somogyi [25]. Lipase activity in the mitochondria, microsomes, and high-speed supernatant fraction from rat liver homogenate, prepared by homogenizing 2 g liver in 10 ml of 0.25 M sucrose containing 0.001 M EDTA, was measured by the method of Guder et al. [9]. Mitochondria and microsomes were suspended in the homogenizing medium (1 ml and 3 ml per g of liver tissue respectively). The *reaction mixture* contained in 1 ml: 0.5 ml of liver fraction, 0.25 ml substrate prepared by homogenizing in a Blender 8 ml corn oil in 12 ml of 10% gum arabic in 0.25 M sucrose, 0.1 ml of 1 M buffer (citrate for pH 5.0 and tris-HCl for pH 8.5). The reaction was carried out at 37° for 30 min. 0.5 ml of this was used for the measurement of free fatty acid released. All animals were sacrificed after fasting for 48 hr or 96 hr.

Table 2
Effects of triamcinolone diacetate and 6-N-2′-O-dibutyryladenosine 3′,5′-(cyclic)-monophosphate on blood glucose levels and hepatic lipase activity at pH 5.0 and 8.5.

Animal	Blood glucose (mg/100 ml blood)	Lipase activity* at pH 4.8	at pH 8.5
Control	110	4.84	2.62
Fasted	78	2.92	1.96
Fasted + hormone (16 hr)	116	4.76	2.53
Fasted + hormone (4 hr)	102	3.02	2.08
Fasted + BcAMP (16 hr)	84	2.84	2.04
Fasted + BcAMP (4 hr)	98	2.98	1.94
Fasted + hormone (16 hr) + actinomycin D	82	3.08	1.90
Fasted + hormone (16 hr) + cycloheximide	80	3.16	2.02
Fasted + BcAMP (4 hr) + cycloheximide	77	2.85	1.88

* Lipase activity is expressed as μmoles of fatty acids released/g of liver/30 min.

Fasting rats were given an intraperitoneal injection of either physiological saline (1.0 ml), triamcinolone diacetate (2.5 mg/100 g body weight), BcAMP (4.0 mg/100 g body weight) actinomycin D (100 μg/100 g body weight) or cycloheximide were injected half an hour prior to the administration of BcAMP or hormone. BcAMP or hormone was administered either 4 hr or 16 hr prior to sacrifice. All animals were sacrificed after 48 hr of fasting.

Table 3
Effects of cyclic AMP, BcAMP, 5′-AMP and triamcinolone diacetate (hormone) on the activities of hepatic lipases occurring in the mitochondria and high-speed supernatant fraction (soluble fraction). The conditions of the experiment were the same as described in table 1.

Addition	*Lipase activity in mitochondria (pH 5.0)	Lipase activity in soluble fraction (pH 8.5)
Control	4.82	2.62
+ BcAMP[a]	4.98	2.58
+ BcAMP[b]	2.56	2.14
+ Cyclic 3′-5′-AMP[a]	4.86	2.68
+ Cyclic 3′-5′-AMP[b]	2.75	2.09
+ 5′-AMP[a]	4.92	2.64
+ 5′-AMP[b]	2.98	2.24
+ Hormone[c]	4.79	2.68
+ Hormone[d]	4.85	2.59

* Lipase activity is expressed as μmoles of fatty acids released/g of liver/30 min.
[a] 0.05 to 1 μmole added
[b] 2.0 to 5.0 μmoles added
[c] 0.02 to 1.0 mg added
[d] 1 to 3 mg added.

4. Discussion

From the results presented in this paper, hepatic lipase, like glucose-6-phosphatase, cannot be considered to be involved in the control of gluconeogenesis, because lipase activity is not increased within 4 to 6 hr after hormone administration, when blood glucose level is elevated and the activities of pyruvate carboxylase, phosphoenolpyruvate kinase and amino-transferases are increased [13, 10]. No increase in glucose-6-phosphatase activity was noticed by Hübener [14] within 4 to 6 hr after cortisone administration of the hormone. In this respect hepatic lipase behaves like glucose-6-phosphatase under hormone action.

The recent findings from several laboratories [15, 16] have indicated that steroid hormones exert their physiological effects in animals by their action at the transcription level, which is believed to be the primary cause of the increases in the activities of several key enzymes through enzyme induction. In the absence of any definite evidence regarding the preference of synthesis of specific RNAs carrying the codes of only those enzymes (proteins) that are involved in the overall process of gluconeogenesis, Tata [15], Tomkins [17] and Garren [16, 18] suggested that thyroid hormone, growth hormone, and glucocorticosteroids (Dexamathasone) can also act at the translation level, in addition to their actions at the transcription level. It has been postulated that the steroidogenic effect of ACTH involves increased formation of cyclic AMP from ATP by adenyl cyclase (due to stimulating action of ACTH on adenyl cyclase) followed by the stimulating action of the cyclic compound on protein biosynthesis at the level of translation of stable mRNA [19–21]. Haynes [22] also demonstrated the ability of cyclic AMP to elicit the steroidogenic action of ACTH.

The amino acid incorporating capacities of microsome-cell sap preparations from the livers of fasting rats before and after treatment with hormone and BcAMP have been investigated and compared in table 4. As previously reported, a drop of 35% (approx.) in amino acid incorporation is observed after 48 hr fasting and this is restored to normal level by the administration of hormone as well as by BcAMP. The stimulating action of hormone is slightly less than that of the cyclic compound. Their actions can be blocked by cycloheximide. Neither cyclic AMP nor BcAMP or 5′-AMP, when added in the range of 100 to 200 μg/ml, has any action on amino acid incorporation by cell sap preparation from fasting (or control) rat livers. Lissitsky et al. [23] found an increase of 50% in amino acid incorporation by thyroid polyribosome preparation in the presence of cyclic AMP (200 μg/ml). An increase of only 20 to 22% in amino acid incorporation by rat liver cell-free system in the presence of cyclic AMP has also been recently reported. Wicks [24] on the other hand, did not notice any stimulating effect of cyclic AMP on protein synthesis by an organ culture system from fetal rat liver. It is not certain whether or not protein synthesis can be stimulated by cyclic AMP (or BcAMP) *in vitro* by cell-free amino acid incorporation system, but it does appear true that cyclic AMP has definitely a stimulating action on protein synthesis *in vivo*.

If the actions of different hormones, as believed, are mediated by cyclic AMP and if the latter acts by virtue of its stimulating action on protein synthesis, as

Table 4
Effects of triamcinolone diacetate and BcAMP on protein synthesis *in vivo* in 48 hr fasting rats.

Animal	TCA-insoluble material (cpm/mg)
Control	986
Fasted	662
Fasted + hormone (4 hr)	932
Fasted + hormone (4 hr) + cycloheximide	634
Fasted + BcAMP (4 hr)	865
Fasted + BcAMP (4 hr) + cycloheximide	649

Hormone and BcAMP were injected 6 hr prior to sacrifice. Other conditions were the same as mentioned in table 2. Amino acid incorporating system was similar to one used previously by Devi and Sarkar [26] and contained in 1 ml: 1 μmole of ATP, 10 μmoles of phosphoenolpyruvate, 40 μg of pyruvate kinase, 0.2 μmoles of GTP, 15 μmoles of $MgCl_2$, 10 μmoles of KCl, 50 μmoles of tris-HCl buffer pH 7.6, and 0.2 ml of the microsomal-cell sap preparation (post mitochondrial supernatant fraction), prepared by homogenizing rat liver in TKM-sucrose buffer (50 mM tris-HCl pH 7.6, 25 mM KCl 5 mM $MgCl_2$ and 0.25 M sucrose) and centrifuging at 20,000 *g* for 10 min in a Spinco (Model L) ultracentrifuge and 0.25 μCi of uniformly labelled leucine. Incubation time was 30 min at 37°. The reaction was stopped by adding an equal volume of cold 10% TCA and 3 ml cold 5% TCA. The samples were prepared for counting according to the procedures previously described [11]. The results are expressed as cpm/mg TCA-insoluble material.

appears to be, it becomes difficult to understand how cyclic AMP can be imagined to be responsible for different physiological effects of different hormones, since the response of polyribosomes to cyclic AMP is non-specific and leads to a general increase in enzyme synthesis. The synthesis of specific enzymes must therefore be preceded by the synthesis of specific RNAs. Cyclic AMP can, at best, be considered, on the basis of present knowledge, as an intracellular regulator of protein biosynthesis and the action of hormones at the translation level is possibly mediated through cyclic AMP.

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